Research Article

Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* in a Tertiary Care Hospital in Central India

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Abstract
Staphylococcus aureus is one of the commonest causes of community acquired and nosocomial infections, and methicillin-resistant strains are increasingly being reported worldwide. This study was carried out to evaluate the prevalence and antimicrobial susceptibility pattern of Methicillin-resistant Staphylococcus aureus (MRSA), isolated from clinical specimens from a tertiary care hospital. The study comprised of 370 strains of Staphylococcus aureus isolated from various clinical specimens using standard microbiological procedures. From these isolates, methicillin resistant strains were screened, and their antimicrobial susceptibility pattern was detected according to CLSI 2017 guidelines. Out of 370 isolates, 140 (37.83%) were found to be methicillin resistant. In this study, vancomycin and linezolid were found to be the only drugs to which all the methicillin resistant Staphylococcus aureus isolates were susceptible. However, vancomycin intermediate and linezolid resistant strains has been reported in some studies. Therefore, all the recommended measures to control the emergence and spread of these strains must be followed strictly in all health care systems.
Keywords: *MRSA, prevalence, antibiotic susceptibility.*

Introduction

Staphylococcus aureus is one of the common organisms found to be associated with both nosocomial and community acquired infections.[1] After penicillin was discovered, the mortality associated with Staphylococcal infections was reduced dramatically. Staphylococcus developed resistant to penicillin soon after its introduction and penicillinase producing strains of staphylococcus species were reported from different parts of world. These Penicillin-resistant strains spread into hospitals and community.[2] Antibiotics that were effective against penicillinase-producing strains like methicillin became the drug of choice for treating *Staphylococcus spp.* related infections. However, soon after its introduction, Methicillin resistant *Staphylococcus aureus* (MRSA) were reported.[3] The first case of MRSA infection was reported from Sydney, Australia in 1965.[4]

Methicillin resistance in *Staphylococcus aureus* is due to the mecA gene that is responsible for resistance to beta lactam antibiotics by encoding the methicillin resistant penicillin binding protein PBP2a. The virulence of MRSA is due to the Panton-Valentine leukocidin factor which is a dermonecrotic and leukocidal toxin.[5]Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) ranges from common skin and soft tissue infections (boils, carbuncles, impetigo, cellulitis) wound infections to the more serious manifestations such as ventilator-associated pneumonias, community-acquired (CA) pneumonia, necrotizing pneumonia, necrotizing fasciitis, and sepsis.[6]

Infected and colonized patients in hospitals mediate the dissemination of MRSA strains, and the main source of transmission of MRSA strains is the health care workers and the hospital staff. This leads to serious endemic and epidemic MRSA infections in hospitals.[7] There are some possible predisposing factors that increase the chance of emergence and spread of MRSA. These factors includes prolonged and repeated hospitalization, indiscriminate use of antibiotics, lack of awareness, intravenous drug abuse, and presence of indwelling medical devices.[8]

Nowadays, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is higher than methicillin susceptible *Staphylococcus aureus* in some countries of America and Asia. The burden of common bacterial infections is higher in low- and middle-income countries, but data about the prevalence of MRSA in these countries are scarce. MRSA infections are associated with increased mortality and also increased costs for health-care systems in developed countries. The spread of MRSA in developing countries can have devastating consequences because of the lack of infrastructure that can provide bacterial identification and antimicrobial susceptibilities and the high cost of antibiotic drugs required to treat severe MRSA infections. [9]

The prevalence of MRSA in India was found to be in range of 21% (moderate) to 45% (high), with overall prevalence of 37.3%.[10]

MRSA strains are difficult to eradicate as they are multidrug-resistant leaving glycopeptides(e.g. Vancomycin) as the drugs of choice.[11] Resistance has been reported to these drugs also from various parts of the country.[9],[10],[12] The knowledge of prevalence of MRSA and their antimicrobial-susceptibility pattern is a must for appropriate treatment of these infections.

The present study was conducted to know the prevalence and antimicrobial susceptibility pattern of MRSA in our hospital.

Material and Methods
Study Design and Study period
A cross sectional study was conducted for a period of one year from July 2017 to June 2018.

Sample Collection and Processing
Staphylococcus aureus isolated from various clinical specimens like pus, urine, sputum, blood, vaginal swab, body fluids etc. were included in the study. All the specimens were collected aseptically in sterile containers and were inoculated on Blood agar and MacConkey agar plates and incubated aerobically at 37^0C for 24 – 48 hours. The isolates were identified as *Staphylococcus aureus* by colony characteristics, gram stain and standard biochemical tests.[13]

Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing was performed by using Kirby Bauer disc diffusion method according to CLSI guidelines 2017.[14] A direct colony suspension equivalent to 0.5 McFarland standard were inoculated on Mueller Hinton agar plates and incubated at 37^0 C for 24 hours. Antibiotics used were erythromycin (15 μg), clindamycin (2 μg), ciprofloxacin (5 μg), cefoxitin (30 μg), tetracycline (30 μg), amikacin (30 μg), gentamicin (10 μg), (co-trimoxazole 25 μg), norfloxacin (10 μg), nitrofurantoin (300 μg), and linezolid (30 μg) (Hi-Media Pvt. Ltd., Mumbai, Maharashtra, India).

Methicillin resistance was detected using surrogate marker cefoxitin disc 30 µg by Kirby Bauer disc diffusion method. A zone of ≤ 21mm was considered as resistant and ≥22 mm as susceptible.

Susceptibility to vancomycin was evaluated by detecting minimum inhibitory concentration (MIC) using E-strips from HiMedia Pvt. Ltd. Isolates with MIC ≤2 µg/ml were considered as sensitive, 4–8 µg/ml were considered as intermediate sensitive and ≥16 µg/ml were considered as resistant.

All the data were compiled using MS EXCEL and presented as tables and percentages.

Results

A total of 370 *Staphylococcus aureus* were isolated from different clinical samples. Pus and wound swabs accounted for the majority of the isolates (67.56%), followed by urine, blood, respiratory samples (sputum, endotracheal tube with tracheal secretion, throat swab etc.), vaginal swab and body fluids (ascitic, pleural, peritoneal etc.). Table 1 shows the distribution of *Staphylococcus aureus* and MRSA isolated from various clinical specimens.

Table 1: Distribution of *Staphylococcus aureus* and MRSA isolated from various clinical specimens

Clinical samples	Total	MRSA	Percentage (%)
Pus and wound swabs	250	100	40.00
Urine	40	14	35.00
Blood	30	10	33.33
Respiratory samples	25	9	36.00
Vaginal swabs	15	4	26.66
Body fluids	10	3	30.00
Total	370	140	37.83
MSSA – Methicillin sensitive *Staphylococcus aureus*. MRSA– Methicillin resistant *Staphylococcus aureus*.			

Overall prevalence of MRSA in the present study was 37.83%. Highest prevalence was seen among MRSA isolated from pus and wound swab (40%).

Table 2 shows resistance of MRSA to individual antimicrobial agents.

Table 2: Resistance of MRSA to individual antimicrobial agents

Antimicrobial agent	MRSA		
	No of strains	Resistant strains	Percentage %
Penicillin	140	140	100
Cefoxitin	140	140	100
Erythromycin[*]	124	75	60.48
Clindamycin[*]	124	38	30.64
Ciprofloxacin	140	100	71.42
Gentamicin	140	59	42.14
Amikacin	140	45	32.14
Tetracycline	140	42	30.00
Co-trimoxazole	140	70	50.00
Nitrofurantoin[**]	16	06	37.50
Norfloxacin[**]	16	09	56.25
Linezolid	140	00	0.00
Vancomycin[***]	140	00	0.00
MRSA – Methicillin Resistant *Staphylococcus aureus*; * not put in urine samples; ** put only in urine samples; ***MIC -Minimum inhibitory concentration			

In our study all MRSA were sensitive to Vancomycin and Linezolid. Maximum resistance was observed against Ciprofloxacin (71.42%), Erythromycin (60.48%), and Co-trimoxazole (50%). Among MRSA isolated from urine, 56.25% were resistant to Norfloxacin and 37.5% were resistant to Nitrofurantoin.

MIC of Vamcomycin in all MRSA strains was in the susceptible range i.e. ≤2 µg/ml.

Discussion

A total of 140 (37.83%) MRSA were isolated from various clinical samples in our study. These results are similar to studies conducted by, Mohanasoundaram *et al.*[15] (39.16%), Abbas *et al.*[4] (40.20%) and Mittal *et al.*[16] (40.38%). Higher prevalence was reported by *Tiwari et al.*[12] (59.3%), Hussian *et al.*[9] (66.25%) and Venkata *et al.*[17] (75.27%). Lower prevalence has been reported by Vijayamohan *et al.*[5] (22.2%), Pai *et al.*[18] (29.1%) and Rajaduraipandi *et al.*[7] (31.1%). This variation in prevalence of MRSA might be because of various factors like infection control practices, health care facilities and antibiotic usage that vary from hospital to hospital.

High prevalence was seen among MRSA isolated from pus and wound swab (40%), followed by

respiratory samples (36%), urine (35%), blood (33.33%), body fluids (30%) and vaginal swab (26.66%). Abbas *et al.*[4] also reported high prevalence among pus sample(43.80% followed by swabs from different sites (41.59%), blood (39.47%), urine (38.73%) and sputum (33.33%). Deepak *et al.*[19] also reported high percentage of MRSA among pus samples 43.10%.

In the present study, all the MRSA strains were susceptible to linezolid. Similar result was obtained by Abbas *et al.*[4] and Arora *et al.*[11] Linezolid was introduced clinically in the year 2000 and a year later in 2001 the first MRSA strain resistant to linezolid was reported. The first Indian case of linezolid resistance was reported from Kashmir in 2011.[9] Recently, linezolid resistant strains have been reported by Hussian *et al.*[9]and Rajkumar *et al.*[10] Linezolid resistance is of concern in all health care settings.

Vancomycin is a drug of choice for infections caused by MRSA. In present study, all MRSA strains were susceptible to vancomycin. Similar result was obtained by Abbas *et al.*[4] and Arora *et al.*[11]In 1996, the first vancomycin-intermediate clinical isolate of S. aureus was isolated in Japan.[9] Recently, vancomycin intermediate strains were reported by Hussian *et al.*[9]and Rajkumar *et al.*[10] Even vancomycin resistant strains were reported by Tiwari *et al.*[12]

In our study maximum resistance was observed against Ciprofloxacin (71.42%), Erythromycin (60.48%) and Co-trimoxazole (50%). These results are in accordance with study conducted by Rajkumar *et al.*[9] Abbas *et al.*[4] reported about 61.19% of MRSA isolates were resistant to erythromycin, 52.73% to ciprofloxacin, 38.80% to clindamycin, 37.81% to gentamicin, 29.35% to co-trimoxazole and 19.40% to tetracycline. Qureshi *et al.*[20] who reported 98.9% resistance to ciprofloxacin and 97.8% to gentamicin, that is much higher when compared to our study. Among MRSA isolated from urine, 56.25% were resistant to Norfloxacin and 37.5% were resistant to Nitrofurantoin. Arora *et al.*[11] reported 53.8 % of

MRSA isolates were resistant to Norfloxacin and 61.5% to Nitrofurantoin.

Conclusion

Multidrug resistant strains of MRSA is increasing day by day. This is worrisome in the present therapeutic scenario especially in the developing countries. There must be regular surveillance of the antimicrobial sensitivity pattern of MRSA in all health care settings. To control the spread of such strains among the patient, basic hygiene measures like hand washing and hand sanitization must be followed strictly. There must be proper antibiotic policy in all hospitals in order to control the emergence of resistant strains. These policies must be followed strictly so that judicial use of antibiotics can be assured and unnecessary and over exposure to the drugs can be avoided.

Vancomycin and Linezolid were found to be 100% sensitive in this study, but vancomycin intermediate and resistant and Linezolid resistant strains have also been reported in some studies. Hence there should be close monitoring of antimicrobial susceptibility pattern of MRSA strains with special reference to vancomycin and linezolid and these drugs should be used judiciously.

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